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Succinate Dehydrogenase (SDH)-deficient Renal Carcinoma: A Morphologically Distinct Entity

A Clinicopathologic Series of 36 Tumors From 27 Patients

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Abstract: Succinate dehydrogenase (SDH)-deficient renal carcinoma has been accepted as a provisional entity in the 2013 International Society of Urological Pathology Vancouver Classification. To further define its morphologic and clinical features, we studied a multi-institutional cohort of 36 SDH-deficient renal carcinomas from 27 patients, including 21 previously unreported cases. We estimate that 0.05% to 0.2% of all

renal carcinomas are SDH deficient. Mean patient age at presentation was 37 years (range, 14 to 76 y), with a slight male predominance (M:F = 1.7:1). Bilateral tumors were observed in 26% of patients. Thirty-four (94%) tumors demonstrated the previously reported morphology at least focally, which included: solid or focally cystic growth, uniform cytology with eosinophilic flocculent cytoplasm, intracytoplasmic vacuolations and inclusions, and round to oval low-grade nuclei. All 17 patients

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who underwent genetic testing for mutation in the *SDH* subunits demonstrated germline mutations (16 in *SDHB* and 1 in *SDHC*). Nine of 27 (33%) patients developed metastatic disease, 2 of them after prolonged follow-up (5.5 and 30 y). Seven of 10 patients (70%) with high-grade nuclei metastasized as did all 4 patients with coagulative necrosis. Two of 17 (12%) patients with low-grade nuclei metastasized, and both had unbiopsied contralateral tumors, which may have been the origin of the metastatic disease. In conclusion, SDH-deficient renal carcinoma is a rare and unique type of renal carcinoma, exhibiting stereotypical morphologic features in the great majority of cases and showing a strong relationship with *SDH* germline mutation. Although this tumor may undergo dedifferentiation and metastasize, sometimes after a prolonged delay, metastatic disease is rare in the absence of high-grade nuclear atypia or coagulative necrosis.

Key Words: *SDHB*, *SDHA*, succinate dehydrogenase, renal carcinoma

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Loss of immunohistochemical (IHC) staining for succinate dehydrogenase subunit B (*SDHB*) has been consistently demonstrated in pheochromocytomas/paragangliomas, gastrointestinal stromal tumors (GISTs), renal carcinomas, and pituitary adenomas arising in the setting of germline mutation of *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2*.^{1–17} Tumors that show loss of staining for *SDHB* (indicating disruption of the mitochondrial complex 2 for any reason, not just *SDHB* mutation) have been termed *succinate dehydrogenase (SDH) deficient*.¹ In addition to absent staining for *SDHB*, tumors associated with *SDHA* mutation also show loss of staining for *SDHA*, whereas tumors associated with germline mutation of *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* show positive staining for *SDHA*.^{1,13,15,18–20}

Because of their strong syndromic and hereditary basis and distinct natural history, SDH-deficient tumors are important to recognize.¹ To date, 53 patients with renal neoplasms arising in the setting of germline *SDH* mutation have been reported (summarized in Supplementary Table 1, Supplemental Digital Content 1, <http://links.lww.com/PAS/A224>).^{4,16,17,21–42} Briefly, 41 cases have been reported arising in the setting of *SDHB* mutation, 5 in the setting of *SDHC* mutation, 3 in the setting of *SDHD* mutation, and none in the setting of *SDHA* mutation. In 4 cases loss of IHC staining for *SDHB* has been reported without follow-up *SDH* mutation testing, but all patients with SDH-deficient renal carcinoma who have undergone complete genetic testing to date have been shown to have germline mutation in one of the *SDH* subunits.

In 2010, we reported that renal carcinomas occurring secondary to *SDH* mutation can be identified by loss of IHC staining for *SDHB*.¹² In 2011, we reported that SDH-deficient renal carcinomas demonstrate distinctive features that allow them to be recognized prospectively and that this morphology can be used to triage IHC staining for *SDHB* as a prelude to formal genetic testing.⁴

Subsequently SDH-deficient renal carcinoma has been recognized as a provisional entity in the recently published 2013 International Society of Urological Pathology (ISUP) Vancouver Classification of renal tumors.⁴³ The entity holds provisional status because relatively few cases have been reported, and therefore experience with the morphologic, immunohistochemical, and clinical features, including long-term outcome, has been limited.

We therefore initiated a broad international collaboration to study these tumors, with the following aims:

- (1) To identify new cases of SDH-deficient renal carcinoma to further expand knowledge and experience with these carcinomas.
- (2) To enable a centralized pathologic review of previously published cases of SDH-deficient renal carcinoma.
- (3) To establish the natural history, clinical features, and prognosis of SDH-deficient renal carcinoma.
- (4) To establish the risk of germline *SDH* mutation associated with SDH-deficient renal carcinoma.
- (5) To estimate the incidence of SDH-deficient renal carcinoma.

METHODS

Case Retrieval and Review

Surgical pathologists with subspecialty interest in urologic pathology or in the pathology of SDH-deficient tumors from 15 institutions in North America, Europe, Asia, and Australia were contacted to submit cases of renal carcinoma occurring in the setting of proven SDH mutation or cases suspected to be associated with SDH deficiency on the basis of morphology, IHC, or a personal or family history of paragangliomas or SDH-deficient GIST. Pathologists were provided with detailed morphologic descriptions, photomicrographs, and published papers,^{4,12} summarizing the previously reported morphology of SDH-deficient renal carcinomas and were asked to review their files for any cases with compatible morphology. Pathologists were asked to provide either a representative block or 10 to 15 unstained slides for centralized pathology review, IHC, and/or genetic testing. Cases from patients previously reported in any form (patients 41 to 53 in Supplementary Table 1, Supplemental Digital Content 1, <http://links.lww.com/PAS/A224>) were also included for review if slides were available, but they were recorded separately to prevent confusion due to double publication of data. For previously published cases, the originating collaborators provided additional clinical follow-up information if available. All submitted cases underwent centralized pathologic review. If the original hematoxylin and eosin sections were unavailable for review (3 cases), the morphologic review was performed by telepathology on scanned whole-slide sections.

Immunohistochemistry

Cases with proven SDH mutation or with compatible morphology underwent IHC analysis for *SDHB* and *SDHA*, which was performed on whole sections with

TABLE 1. Clinical, Pathologic, and IHC Features of Previously Unreported SDH-deficient Renal Carcinomas

	Location	Age	Sex	Size (mm)	Surgery	Stage	ISUP Grade	Necrosis	Status	Follow-up (mo)
1	Right	35	M	75	Partial nephrectomy	2 (T2AN0)	2	No	ANED	3
2	Left	76	F	25	Partial nephrectomy	1 (T1AN0)	2	No	ANED	0
3	Right	32	F	68	Nephrectomy	1 (T1BN0)	2	No	ANED	8
4			M						ANED	
	Right	34		50	Wedge	1 (T1BN0)	2	No	ANED	53
	Left	35		75	Nephrectomy	2 (T2AN0)	4†	No	ANED	50
	Left	35		47	Nephrectomy	1 (T1BN0)	2	No	ANED	50
	Left	35		40	Nephrectomy	1 (T1AN0)	2	No	ANED	50
5			M							
	Left	45		50	Nephrectomy	1 (T1BN0)	2	No	ANED	27
	Left	45		41	Nephrectomy	1 (T1BN0)	2	No	ANED	27
	Right	45		7	Wedge	1 (T1AN0)	2	No	ANED	25
6	Left	43	F	38	Nephrectomy	1 (T1AN0)	2	No	ANED	38
7	Right	31	F	35	Partial nephrectomy	1 (T1AN0)	2	No	ANED	1
8	Right	16	M	45	Nephrectomy	1 (T1BN0)	2	No	ANED	1
9	Right	46	M	85	Nephrectomy		3	No	ANED	4
10	Right	30	F	90	Nephrectomy (left kidney mass found, 362 mo not resected)	p2 (T2AN0)	2	No	AWD vertebral met 362 mo	368‡
11*			M							
	Left	14					2	No	AUNDS	240
	Right	18					2	No	AUNDS	192
	Right	18					2	No	AUNDS	192
12*	?	44	F				4†	Yes	DOD	12
13	Right	57	M	60	Partial nephrectomy (left kidney 70 mm mass unresected)	p3 (T3aNO)	2	No	DOD Liver met 4 mo	10
14	Right	54	M	28	Partial nephrectomy	1 (T1aNO)	2	No	ANED	5

*Case 12 is the mother of case 11.

†Tumor showed areas of high-grade transformation in direct continuity with lower-grade areas.

‡At the time of presentation with metastatic disease the patient was found to have a metachronous tumor in the left kidney (not biopsied). Therefore the vertebral metastasis may represent either a delayed metastasis from the original primary or metastasis from metachronous disease.

ANED indicates alive no evidence of disease; AUNDS, alive with unknown disease status; AWD, alive with disease; DOD, dead of disease.

mouse monoclonal antibodies against SDHB (ABCAM ab14714, clone 21A11, dilution 1 in 100) and SDHA (Mitosciences Abcam MS204, clone 2E, dilution of 1 in 1000), -detailed methods previously described.^{3,4,6,12,13,15,18} Cases with definite granular cytoplasmic staining were classified as SDHB/SDHA positive. Cases with absent cytoplasmic staining in the presence of an internal positive control of non-neoplastic cells were classified as negative. If there was negative staining in the neoplastic cells but no internal positive control in the non-neoplastic cells, the staining was considered indeterminate and repeated. A panel of IHC markers commonly used in urologic pathology (PAX8, AMACR, CD10, c-KIT, AE1/AE3, CK8/18, cytokeratin 7, cytokeratin 20, and EMA) was also performed if tissue was available.

Molecular Methods

DNA Extraction

DNA from formalin-fixed paraffin-embedded (FFPE) tumor tissue was extracted using QIAasympy DNA Mini Kit (Qiagen, Hilden, Germany) on an automated extraction system (QIAasympy SP; Qiagen) according to manufacturer's supplementary protocol for FFPE samples (Purification of genomic DNA from FFPE tissue using the QIAamp DNA FFPE Tissue Kit and Deparaffinization Solution). Concentration and purity of

isolated DNA was measured using NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE). DNA integrity was examined by amplification of control genes in a multiplex polymerase chain reaction (PCR).

Analysis of SDHB Gene Mutation

Mutational analysis of complete CDS and exon-intron junctions of the *SDHB* gene was performed using PCR and direct sequencing. Briefly, 100 ng DNA was added to a reaction mixture consisting of 12.5 µL of FastStart PCR Master (Roche Diagnostic, Mannheim, Germany), 10 pmol of forward and reverse primers, and distilled water up to 25 µL. The amplification program consisted of denaturation at 95°C for 9 minutes, 35 cycles of denaturation at 95°C for 1 minute, annealing 62°C for 1 minute, and extension at 72°C for 1 minute. The program was terminated by incubation at 72°C for 7 minutes. The PCR products were separated by electrophoresis through a 2% agarose gel.

Successfully amplified PCR products selected for sequencing analysis were purified with magnetic particles Agencourt AMPure (Agencourt Bioscience Corporation, A Beckman Coulter Company; Beverly, MA), both side sequenced using Big Dye Terminator Sequencing kit (Applied Biosystems) and purified with magnetic particles Agencourt CleanSEQ (Agencourt Bioscience Corporation, A Beckman Coulter Company), all according to the

TABLE 1. (continued)

	Mutation	SDHB	SDHA	PAX8	AMACR	CD10	c-KIT	EMA	CK7	CK20	AE1/AE3	CK8/18
1	SDHB [c.137G > A,pArg46Gln]	Neg	Pos	Pos	Neg	Focal	Neg	Focal	Neg	Neg	Neg	Neg
2	SDHB [c.725G > A,p.Arg242His]	Neg	Pos	Pos	Pos	Neg	Neg	Focal	Neg	Neg	Neg	Neg
3	SDHB [c.423 + 1G > A] Splice	Neg	Pos	Pos	Neg	Pos	Neg	Pos	Neg	Neg	Neg	Neg
4	SDHB exon 3 deletion	Neg	Pos	Pos	Focal	Focal	Neg	Focal	Neg	Neg	Neg	Neg
		Neg	Pos	Pos	Neg	Focal	Neg	Focal	Neg	Neg	Neg	Neg
		Neg	Pos									
		Neg	Pos									
5	SDHB [c.423 + 1G > A] Splice	Neg	Pos	Pos	Pos	Neg	Neg	Focal	Neg	Neg	Neg	Neg
		Neg	Pos	Pos	Pos	Focal	Neg	Focal	Neg	Neg	Pos	Pos
		Neg	Pos	Pos	Pos	Neg	Neg	Focal	Neg	Neg	Neg	Neg
6		Neg	Pos	Pos	Pos	Neg	Neg	Focal	Neg	Neg	Neg	Neg
7	SDHB [c.338G > A, p.Cys113Tyr]	Neg	Pos	Pos	Pos	Pos	Focal	Focal	Neg	Neg	Neg	Neg
8		Neg	Pos	Pos	Neg	Focal	Neg	Focal	Neg	Neg	Neg	Neg
9		Neg	Pos	Pos	Pos	Focal	Neg	Focal	Neg	Neg	Neg	Neg
10	SDHB [c.423 + 1G > A] Splice	Neg	Pos	Pos	Pos	Neg	Neg	Focal	Neg	Neg	Focal	Focal
11*		Neg	Pos	Pos	Neg	Focal	Neg	Focal	Neg	Neg	Neg	Neg
		Neg	Pos									
		Neg	Pos									
12*		Neg	Pos	Focal	Pos	Neg	Neg	Pos	Neg	Neg	Pos	Pos
13	SDHB [c.749C > A, p.Thr250Lys]	Neg	Pos	Pos	Pos	Focal	Neg	Pos	Neg	Neg	Pos	Pos
14		Neg	Pos	Focal	Focal	Focal	Neg	Focal	Focal	Neg	Pos	Pos

manufacturer's protocol. Samples were then run on an automated sequencer ABI Prism 3130xl (Applied Biosystems) at a constant voltage of 13.2 kV for 20 minutes. DNA sequences were compared with the reference sequence (<http://www.ncbi.nlm.nih.gov>) by the online program BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Incidence Assessment

To assess the incidence of SDH-deficient renal carcinoma in an unselected population, the computerized database of the Department of Anatomical Pathology Royal North Shore Hospital, Sydney, Australia was searched for all primary renal neoplasms resected between 1998 and 2013, with material available in archived FFPE blocks (excluding consultation cases). Similar assessments were made using the database of renal tumors, collected between 2000 and 2013 in the Department of Pathology and Laboratory Medicine of the Calgary Laboratory Services and University of Calgary, Alberta, Canada and for the tumors collected between 2003 and 2013 in the renal tumor registry at the Department of Pathology, Charles University, Pilsen, Czech Republic. The original slides were reviewed explicitly in search of cases with morphology considered compatible with proven cases of SDH-deficient renal carcinoma.

Representative areas of each tumor from the Royal North Shore Hospital cohort were also marked for tissue microarray (TMA) construction. The TMA was constructed with duplicate 1-mm-thick cores of neoplastic

tissue from all available cases, and this TMA was evaluated by IHC for SDHB.

RESULTS

Clinical Features

We identified 21 previously unreported SDH-deficient renal carcinomas from 14 patients. The clinical and IHC features are summarized in Table 1. Briefly, the mean age at presentation with a renal tumor was 39.8 years (range, 14 to 76 y; median 43.5 y), with a slight male predominance (M:F = 1.3:1). At presentation all tumors with known size and stage were confined to the kidney, with an average size of 51 mm (range, 7 to 90 mm). The mean follow-up from initial presentation was 55 months (4.6 y) with a range of 0 to 368 months (30.7 y). Three of the 14 patients (21%) were known to have developed metastatic disease. One of these patients died 12 months after presentation (stage at presentation unknown). The other 2 patients with metastasis had unbiopsied neoplasms in the contralateral kidney, which were identified at the time of presentation with metastasis. One of these patients developed liver metastasis, proven by fine-needle aspiration, 4 months after partial nephrectomy and died of disease 10 months after surgery. The other patient developed vertebral metastases, confirmed by core biopsy, 362 months after nephrectomy and is alive with disease 368 months (30.7 y) after the initial presentation.

TABLE 2. Clinical, Molecular, and IHC Details of Previously Published Patients With Material Available for Pathologic Review

	Prev. Pub.	Location	Age	Sex	Size (mm)	Surgery	Stage	ISUP Grade	Necrosis	Follow-up (mo)	Status
15	16	Bilateral	27	M	41	Biopsy of met only	Stage 4	3	Yes	30	DOD
16	4,12	Right	21	F	22	Wedge	1 (T1ANO)	2	No	84	ANED
17	4,12	Left	28	M	29	Nephrectomy	1 (T1A)	2	No	48	ANED
18	4,12	Left	22	M	100	Nephrectomy	Stage 4	4	Yes	12	DOD
19	4,37,42	Left	58	F	78	Nephrectomy	2 (T2AN0)	2	No	24	ANED
20	17	Right	22	F	65	Nephrectomy	1 (T1BN0)	2	No	160	ANED
21	41			M		Nephrectomy			No		
		Left	25		90	Nephrectomy	2 (T2AN0)	2	No	72	ANED
		Left	25		28	Nephrectomy	1 (T1AN0)	3	No	72	ANED
		Right	31		13	Radio-frequency ablation	1 (T1AN0)	2	No	0	ANED
22	41	Right	23	M	25	Nephrectomy	1 (T1AN0)	2	No	60	ANED
23	41	Left	36	M	130	Nephrectomy	2 (T2BN0)	3	No	132	AWD Spleen met 66 mo, liver met 10 mo
24	43		40	M			4	3	No		Adrenal metastasis
25	43		35	M				3	Yes		Retroperitoneal node metastasis
26	43		44	M			4	3	No		Adrenal metastasis
27	43	Right	59	F	70		2 (T2AN0)	2	No		AUNDS

ANED indicates alive with no evidence of disease; AUNDS, alive with unknown disease status; AWD, alive with disease; DOD, dead of disease.

Fifteen SDH-deficient renal carcinomas from 13 previously published patients^{4,12,16,17,40,42} were available for central pathologic review (summarized in Table 2, with updated survival data). These cases also showed a male predisposition (M:F = 2.3:1) but otherwise demonstrated similar demographic features with mean age at initial presentation of 33.8 years. Two of these patients died of metastatic disease (at 12 and 30 mo, respectively), and 1 patient was alive with metastatic disease 132 months (11 y) after presentation. Three other previously reported patients were also known to have developed metastatic disease (2 to the adrenal gland, 1 to retroperitoneal lymph node) but lacked further follow-up.

When both the previously reported (Table 2) and novel patients (Table 1) were combined, the mean age at first presentation was 37 years (range, 14 to 76 y). There was a slight male predominance (M:F = 1.7:1). There were 4 patients with multifocal tumors in the same kidney, and bilateral neoplasms were present in 7 of 27 (26%) patients.

The incidence of synchronous or metachronous GIST and pheochromocytoma/paraganglioma as well as the family history of renal carcinoma, GIST, and pheochromocytoma/paraganglioma are presented in Table 3. Briefly, 4 of 27 (15%) patients also had SDH-deficient GISTs, and 4 of 27 (15%) patients developed paragangliomas. Five patients (19%) had first-degree relatives with renal carcinoma and 1 patient a second-degree relative. There were 5 first-degree and 2 second-degree relatives with pheochromocytoma/paraganglioma and 1 first-degree relative with SDH-deficient GIST. Two patients also had incidental small renal angiomyolipomas resected. The angiomyolipoma from patient 6 was 10 mm in diameter, and the angiomyolipoma from patient 9 was

3 mm in diameter. The angiomyolipoma from patient 9 was available for IHC and demonstrated positive staining for SDHB. Neither patient with angiomyolipomas was known to have tuberous sclerosis complex.

Pathologic Features

Centralized pathologic review was undertaken on 36 available SDH-deficient renal carcinomas from 27 patients. Macroscopic descriptions of the tumors were not always detailed or available, but in all cases with gross description, the tumors were characterized as well circumscribed with a tan to red cut surface. Some of the tumors were noted to demonstrate cystic change. Although this cystic change was sometimes striking (Fig. 1A), this was not a constant feature, and the majority of tumors were solid (Fig. 1B).

Histologically, the dominant morphology was as previously described⁴ and was found at least focally in 34 tumors from 24 patients. This morphology is illustrated in Figures 2–5, and whole-slide scanned images from all tumors are available for review at <http://www.cancerdxpathology.org.au>. Briefly, the tumors were well circumscribed or demonstrated coarse lobulation, with a pushing border sometimes associated with a pseudocapsule (Figs. 2A, B). Cystic change in the form of microcysts and macrocysts was commonly appreciated histologically, and these cysts usually contained pale eosinophilic fluid (Figs. 2C, D). In a few tumors the stroma showed areas of prominent myxoid change or hyalinization. The neoplastic cells were cuboidal to oval with round nuclei and inconspicuous nucleoli, consistent with an ISUP nucleolar (nuclear) grade 2 in 26 cases (Fig. 3). The nuclei were grade 3 in 7 cases and grade 4 in 3 cases (all of which demonstrated at least focal sarcomatoid change).

TABLE 2. (continued)

	Germline Mutation	SDHB	SDHA	PAX8	AMACR	CD10	c-KIT	EMA	CK7	CK20	AE1/AE3	CK8/18
15	SDHB c.88delC	Neg	Pos	Pos	Neg	Focal	Neg	Pos	Focal	Neg	Pos	Pos
16	SDHB c.268C > T	Neg	Pos	Pos	Neg	Neg	Neg	Focal	Neg	Neg	Neg	Neg
17	SDHB c.166-170 delCCTCA	Neg	Pos	Pos	Pos	Focal	Neg	Focal	Neg	Neg	Focal	Neg
18	SDHB c.423 + 1G > A	Neg	Pos	Pos	Focal	Focal	Neg	Pos	Pos	Neg	Pos	Pos
19	SDHB c.72 + 1G > T	Neg	Pos	Pos	Pos	Focal	Neg	Neg	Neg	Neg	Neg	Neg
20	SDHC c.380A > G	Neg	Pos	Pos	Neg	Neg	Neg	Focal	Neg	Neg	Neg	Neg
21	SDHB c.3G > A	Neg	Pos									
		Neg	Pos									
		Neg	Pos									
22	SDHB c.3G > A	Neg	Pos	Pos	Pos	Focal	Neg	Focal	Neg	Neg	Neg	Neg
23	SDHB exon 3 deletion	Neg	Pos	Pos	Pos	Focal	Neg	Focal	Neg	Neg	Neg	Neg
24		Neg	Pos									
25		Neg	Pos									
26		Neg	Pos									
27		Neg	Pos									

In most tumors the nuclear chromatin commonly had a dispersed quality reminiscent of cells with neuroendocrine differentiation. The cell borders were sometimes indistinct. The cytoplasm was eosinophilic or flocculent but not truly oncocytic (Fig. 3). Tumor cells demonstrated a variably solid or nested architecture, and sometimes nests of tumor cells surrounded cystic spaces imparting a pseudoglandular appearance.

The most constant and distinctive histologic feature was the presence of cytoplasmic vacuoles and inclusion-like spaces (Figs. 3C, D). These contained either pale eosinophilic fluid or flocculent material. In most cases, these inclusions were readily identified throughout the tumor, but in some cases, particularly in those with higher-grade nuclei, these cytoplasmic inclusions were subtle and were only identified focally after a thorough search of multiple sections (Fig. 5). Non-neoplastic tubules or glomeruli were frequently entrapped at the periphery of the neoplasm (Fig. 4). Intratumoral mast cells were commonly highlighted with c-KIT IHC but were not appreciable as a conspicuous finding on routine hematoxylin and eosin sections. Allowing for the secondary effects of the tumor, the adjacent non-neoplastic kidney was normal, and no dysplastic or precursor lesions were identified in the adjacent renal parenchyma.

In the 5 tumors with ISUP nucleolar (nuclear) grade 3 nuclei, which were still recognizable as SDH-deficient renal carcinomas, in addition to prominent nucleoli, the neoplastic cells in the higher-grade areas acquired darker and coarser chromatin and more dense eosinophilic (rather than flocculent) cytoplasm. The nuclei in these areas were about 2 times larger than the nuclei in low-grade areas and demonstrated oval to slightly elongated shape, with irregular nuclear outlines. In some areas these tumors lost their nested architecture and commonly grew as solid sheets, occasionally with a very focal abortive papillary architecture.

Three cases demonstrated frank sarcomatoid transformation, with ISUP nucleolar (nuclear) grade 4.

The sarcomatoid areas were composed of pleomorphic spindled cells essentially indistinguishable from other high-grade sarcomatoid renal carcinomas. In 2 of the cases with sarcomatoid change, the sarcomatoid areas were in direct continuity with areas showing the stereotypical low-grade morphology (including ISUP nucleolar [nuclear] grade 2 nuclei), indicating true dedifferentiation rather than the existence of a different tumor type. In the other case with areas of sarcomatoid transformation, the entire tumor was high grade (either grade 3 or grade 4 nuclei). However, even in this case intracytoplasmic inclusions, albeit subtle, were identified after a search of multiple slides.

Although fibrosis, hyalinization, and hemorrhage were not uncommon, true coagulative necrosis was only found in 4 tumors—all ISUP nucleolar (nuclear) grade 3 or 4.

Only 2 of 36 (6%) cases lacked any areas with typical morphologic features or cytoplasmic inclusions and would not have been recognizable as SDH-deficient renal carcinomas on the basis of morphology. These cases, illustrated in Figure 6, were previously reported by Miettinen et al⁴² and identified by screening a large cohort by IHC rather than triaging IHC on the basis of morphology.⁴² In 1 case, the morphology was that of a typical clear cell renal carcinoma, ISUP nucleolar (nuclear) grade 3. In this case, only 1 block was available for review. In the second case, the morphology was in keeping with papillary renal carcinoma type 2, ISUP nucleolar (nuclear) grade 3. In this case, 4 blocks were available for review, all of which demonstrated similar histology.

Immunohistochemistry

All cases demonstrated negative staining for SDHB in all neoplastic cells (which was considered an inclusion criterion for the study). All cases also showed preserved positive staining for SDHA. At least focal positive staining for PAX8 was found in all cases. All but 1 case (96%) demonstrated at least focal reactivity for EMA,

TABLE 3. Personal and Family History of Renal Carcinoma, SDH-deficient GIST, Pheochromocytoma, Paranglioma

	Total Number of RCCs	Family History RCC	Personal History SDH-deficient GIST	Family History SDH-deficient GIST	Personal History Pheochromocytoma or Paranglioma	Family History Pheochromocytoma or Paranglioma
1	1	No	No	No	No	No
2	1	Unknown	Unknown	Unknown	Unknown	Unknown
3	1	No	No	No	No	No
4	4	No	No	No	No	No
5	3	No	Age 45 y	No	No	No
6	1	No	No	No	No	No
7	1	No	No	No	No	No
8	1	Unknown	No	Unknown	No	Unknown
9	1	No	No	No	No	No
10	2*	No	No	No	No	Nephew, paraganglioma age 23 y
11	3	Son of patient 12	No	No	Paranglioma, age 14 y	No
12	2	Mother of patient 11	Unknown	Unknown	Unknown	Mother of patient 11
13	2*	No	No	Unknown	No	Unknown
14	1	No	Age 54 y	No	No	No
15	2†	No	No	No	No	No
16	1	No	No	No	No	Mother, paragangliomas × 3 (age 43, 45, 45 y)
17	1	Maternal aunt, age 34 y	No	No	Paranglioma age 28 y	No
18	1	No	No	Brother age 44 y	No	Nephew, paraganglioma age 19 y
19	1	Sister Renal Carcinoma	No	No	No	Brother, paraganglioma age 44 y
20	1	No	Age 12 and 33 y	No	No	Daughter metastatic pheochromocytoma
21	3	Brother of patient 22	No	No	Paranglioma, age 25 y	No
22	1	Brother of patient 21	No	No	No	Brother of patient 21
23	1	No	No	No	Paranglioma age 30 y Paranglioma age 34 y	No
24	1	Unknown	Unknown	Unknown	Unknown	Unknown
25	1	Unknown	Unknown	Unknown	Unknown	Unknown
26	1	Unknown	Unknown	Unknown	Unknown	Unknown
27	1	Unknown	Age 19	Unknown	Unknown	Unknown

*This number includes a presumed SDH-deficient renal carcinoma identified by imaging in the contralateral kidney but not biopsied or resected.

†This number includes bilateral renal tumors identified on imaging but not biopsied or resected.

which was often quite limited, in some cases involving <1% of neoplastic cells, and commonly restricted to the apical border of cells. Only 3 of 25 cases (12%) demonstrated positive staining for CK7, and this staining was focal in 2 cases. Immunoreactivity for other markers was not specific. It is noteworthy that 68% of the cases demonstrated completely negative staining for all cytokeratins. IHC for c-KIT was negative in 96% of cases but did highlight scattered intratumor mast cells in many tumors.

Genetic Testing

Of the previously reported cases, 9 had undergone germline molecular testing and were found to harbor a pathogenic mutation in *SDHB* (8 cases) or *SDHC* (1 case)—mutation data previously reported.^{4,8,12,16,17,40} Of the previously unpublished cases, genetic testing was performed for *SDHB* in 8 patients, and in all of them a

pathogenic germline mutation was identified. That is all 17 patients with SDH-deficient renal carcinoma who have undergone testing were found to harbor a germline mutation of 1 of the components of the mitochondrial complex 2 (16 *SDHB*, 1 *SDHC*, and none in *SDHA* or *SDHD*).

Morphologic Predictors of Metastasis

A total of 9 patients with pathologic material available for histologic review developed metastatic disease (6 previously reported and 3 new patients). Four of these patients died of metastatic disease at a mean of 18 months after initial presentation, all of whom had an IS-UP nucleolar (nuclear) grade of 3 or 4 at presentation, and 3 of whom had coagulative necrosis. The other patient with coagulative necrosis was known to have metastatic disease but had no further follow-up information available. Two patients were alive with metastatic disease, 132

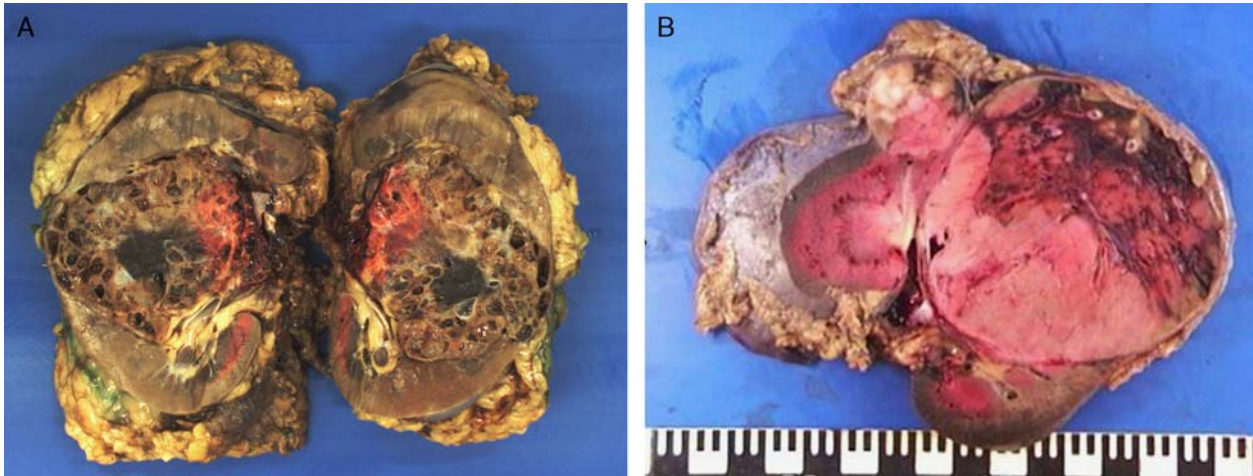


FIGURE 1. Although many of the tumors demonstrated cystic change, which was often profound (A, 85 mm tumor from the right kidney of patient 9), this was not a constant finding, and some neoplasms were solid (B, 2 solid tumors, 90 and 28 mm, from the left kidney of patient 21).

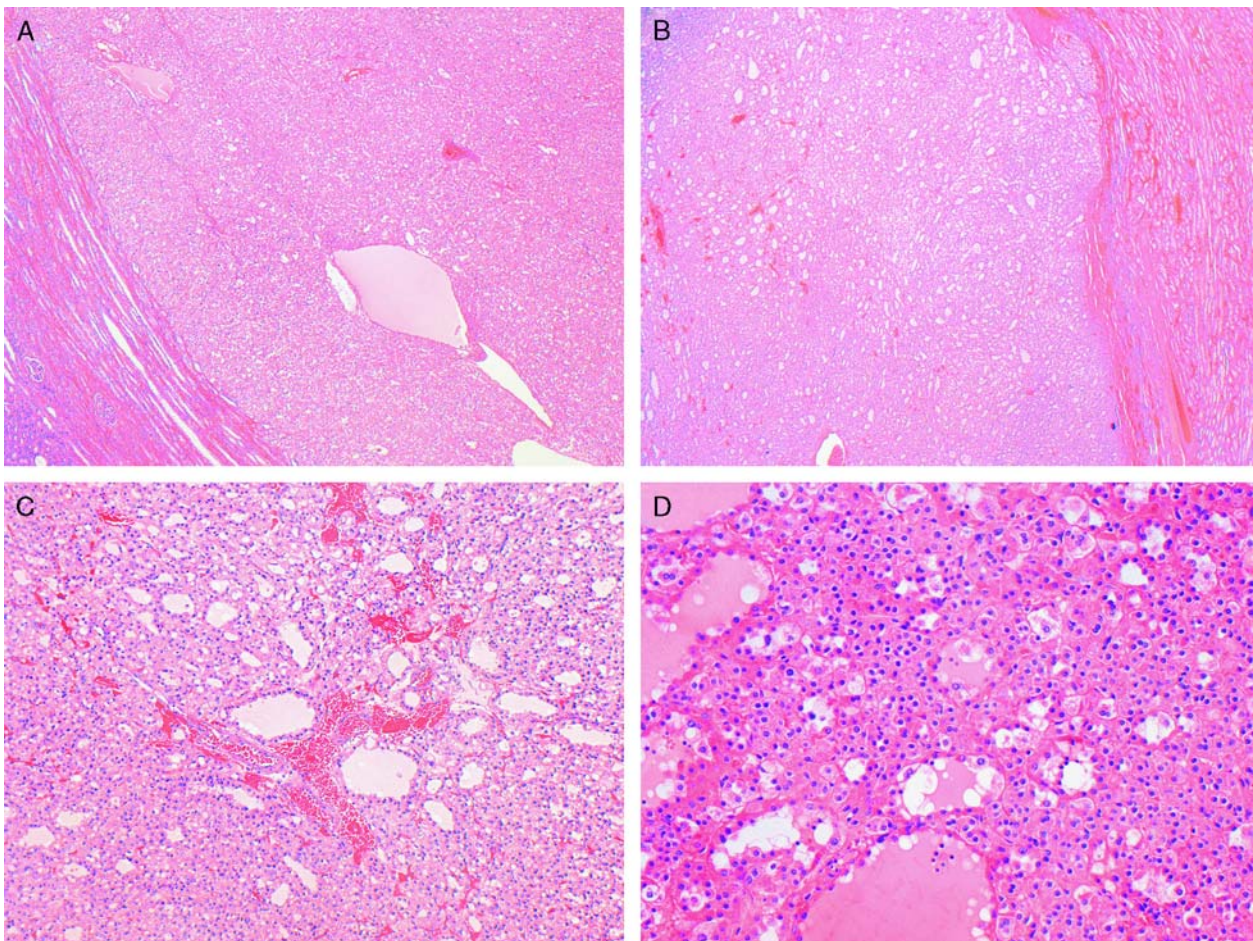


FIGURE 2. The tumors were well circumscribed (A) and only occasionally separated from the adjacent kidney by a pseudocapsule (B). C and D, Cystic change was commonly appreciated histologically, and the cystic spaces contained pale eosinophilic fluid (hematoxylin and eosin).

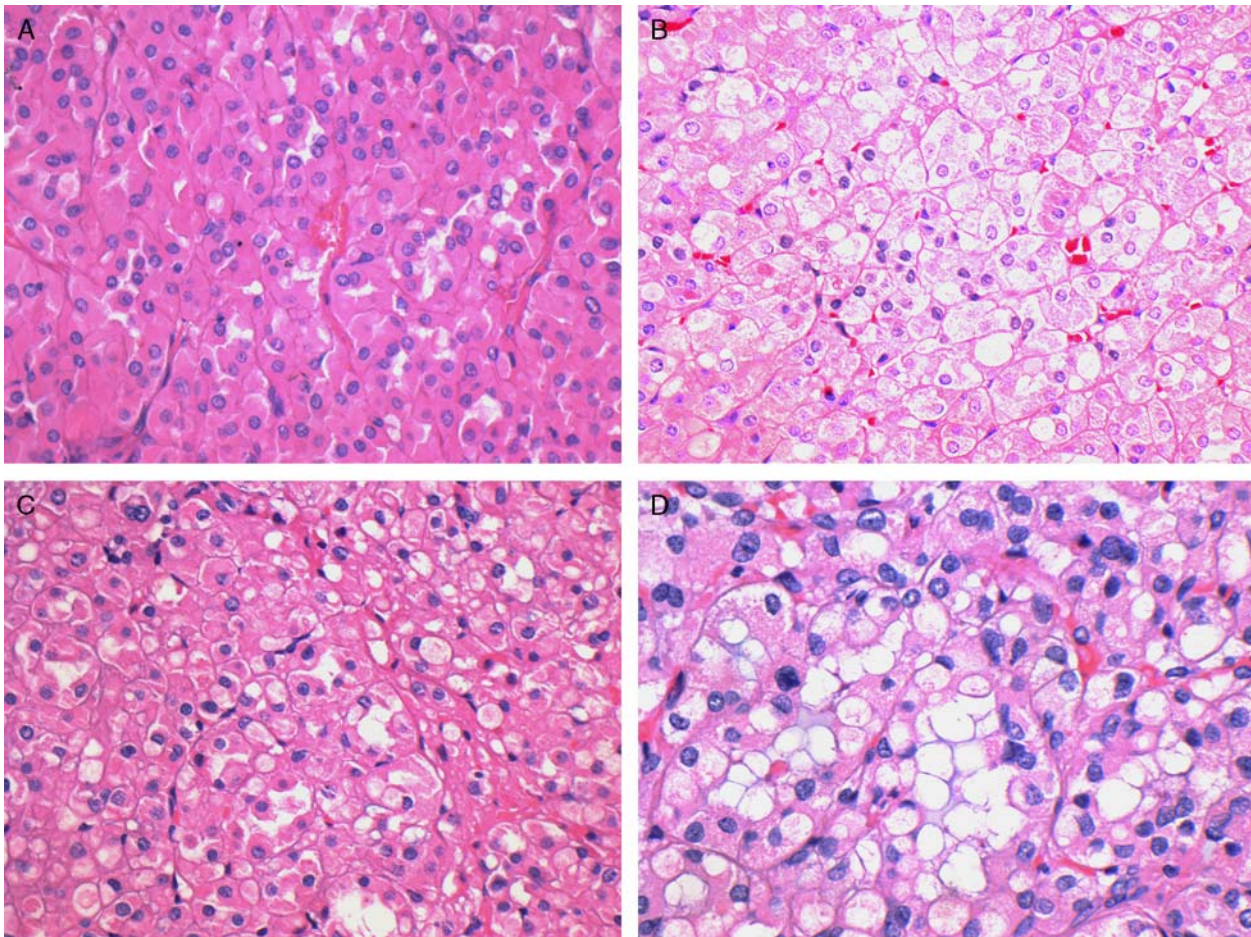


FIGURE 3. The tumor cells had eosinophilic cytoplasm but lacked the granularity associated with true oncocytes. In some cases the eosinophilic cytoplasm was dense (A), but in most cases (B and C) it had a pale and wispy, almost flocculent, appearance. In some tumors (D) the combinations of flocculent cytoplasm and frequent intracytoplasmic inclusions imparted a bubbly appearance to many of the tumor cells (hematoxylin and eosin).

months (11 y) and 368 months (30.7 y) after initial presentation. One of these 2 patients showed increased cytologic atypia and an ISUP nucleolar (nuclear) grade 3 but lacked frank sarcomatoid change and subsequently developed biopsy-proven metastases in the spleen at 66 months (5.5 y) and the liver at 108 months (9 y) after initial presentation. The other patient showed only typical low-grade features in the initial resection with an ISUP nucleolar (nuclear) grade of 2, but then developed biopsy-proven vertebral metastasis 30 years later. The metastasis showed increased cytologic atypia with an ISUP nucleolar (nuclear) grade 3 and an abortive papillary architecture but lacked sarcomatoid differentiation (Fig. 7). Importantly, at the time of diagnosis of the metastasis, this patient was found to have a solid tumor on diagnostic imaging in her contralateral kidney. Unfortunately, this tumor was not biopsied or resected, and the origin of the metastasis, either from the original SDHB tumor or from the metachronous neoplasm in the contralateral kidney,

could not be established with certainty. Both patients with exclusively variant morphology (illustrated in Fig. 6) developed metastatic disease, but no further follow-up information was available.

Estimated Incidence

The review of consecutive unselected cases from the Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia identified 420 renal neoplasms. None of these tumors demonstrated morphologic features of SDH-deficient renal carcinoma, and IHC for SDHB, performed on a TMA, was positive in all cases, suggesting that the incidence in a truly unselected group of primary renal carcinomas is <1 in 420 (0.2%). The database from the Rockyview Hospital (Calgary Laboratory Services and University of Calgary) included 1750 in-house resected renal tumors. All renal neoplasms, reported as “*unclassified*” or “*oncocytic*” were reviewed, and 2 cases were identified on the basis of morphology,

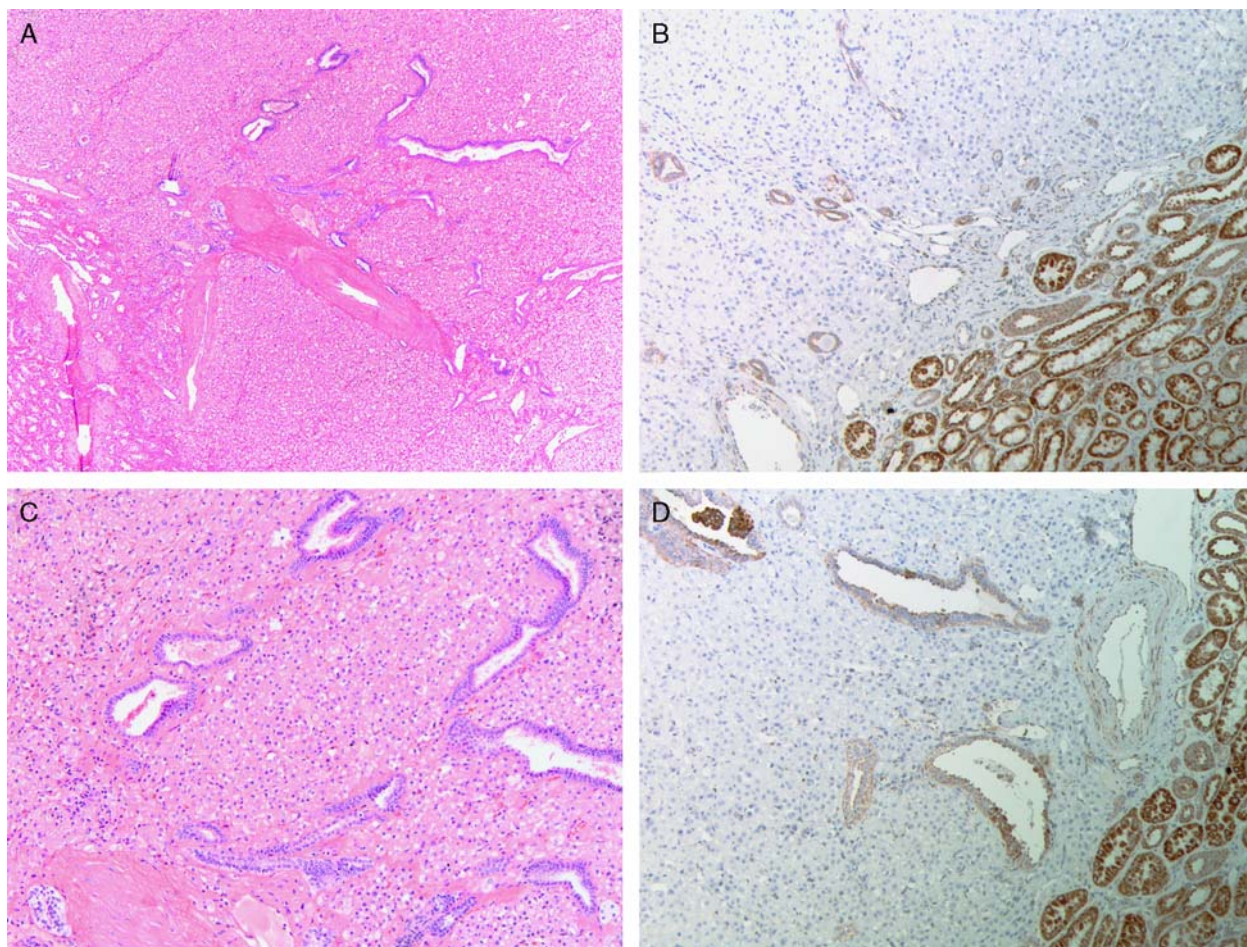


FIGURE 4. Serial sections stained with hematoxylin and eosin (A and C) and SDHB IHC (B and D). Frequently entrapped benign tubules were noted at the edge of the tumors. SDHB IHC demonstrates positive staining in the internal controls (including the entrapped benign tubules) but all the neoplastic cells are negative.

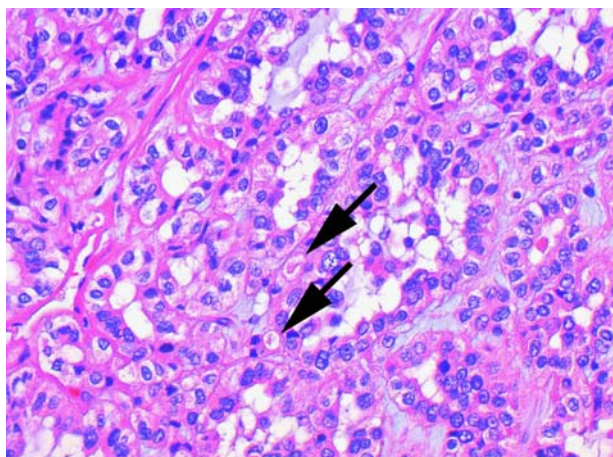


FIGURE 5. In this case with higher-grade nuclear features and early dedifferentiation, the intracytoplasmic inclusions are more subtle (arrows) and were identified only after a careful search (hematoxylin and eosin).

with an estimated overall incidence of 0.1%. The morphologic review of the renal tumor registry at the Department of Pathology, Charles University, Pilsen, Czech Republic, identified only 1 case from 2004 locally resected tumors, with an estimated incidence of 0.05%.

DISCUSSION

SDH-deficient renal carcinoma has recently been accepted as a provisional entity in the 2013 ISUP Vancouver Classification. However, reflecting its rarity, published experience with this tumor has been limited. To substantiate its distinctive morphologic and clinical features, the prognosis, and the genetic associations of SDH-deficient renal carcinoma and to estimate its incidence, we evaluated a multi-institutional cohort of 36 SDH-deficient renal carcinomas from 27 patients, including 21 previously unreported cases.

This study confirmed that the previously reported distinctive morphologic features of SDH-deficient renal

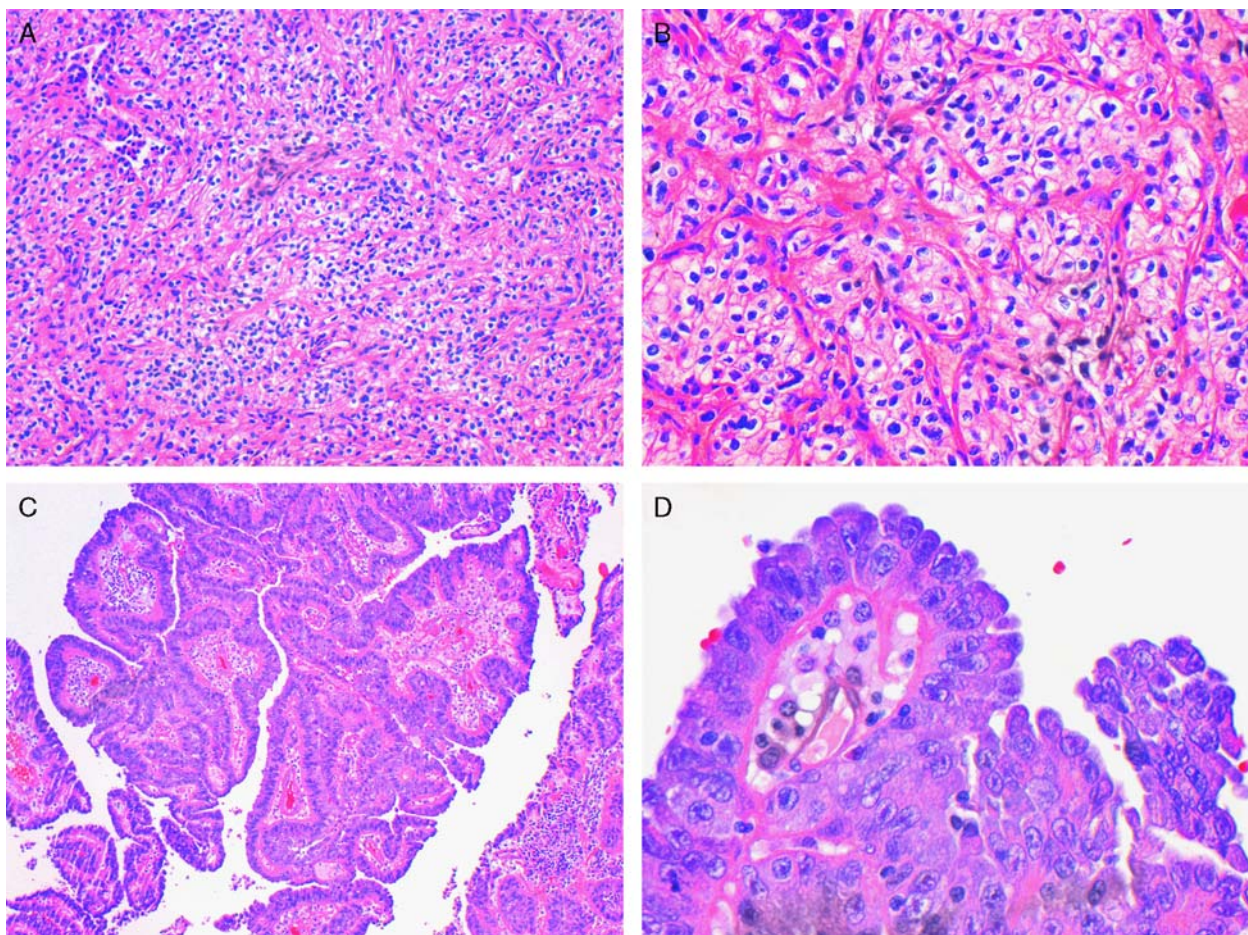


FIGURE 6. Cases with variant morphology. One case demonstrated morphology reminiscent of conventional clear cell renal carcinoma of ISUP nucleolar (nuclear) grade 3 (A and B). C and D, A second case demonstrated a papillary architecture with prominent nucleoli, reminiscent of type 2 papillary renal carcinoma (hematoxylin and eosin).

carcinoma are highly specific for the diagnosis. That is, all the cases with the typical morphology demonstrated negative staining for SDHB. Therefore morphology should be considered the primary screening test to identify SDH-deficient renal carcinoma in routine practice. However, we caution that the study was not intended or designed to demonstrate that all renal carcinomas arising in the context of *SDH* mutation will show this morphology. That is many cases reported in this series were first identified primarily on the basis of morphology, and only selected cases with compatible morphology then underwent screening IHC. Therefore, there may be a selection bias in this series toward cases with typical morphologic features. It is therefore worth noting that 2 (6%) cases from this series (both identified by IHC screening of large cohorts) lacked this distinctive morphology, and in other cases (particularly those with high ISUP nuclear [nucleolar] grade) this morphology was only a focal finding and may not be appreciated in routine clinical practice. Therefore, in addition to performing SDHB IHC on cases with compatible morphology, regardless of

age or clinical features, we would also recommend that screening IHC be considered for other cases with suggestive clinical features (for example, multifocality, onset at a young age, or a personal or family history of renal carcinoma, pheochromocytoma/paraganglioma, gastric GIST, or pituitary adenoma).

We would estimate the true incidence of SDH-deficient renal carcinoma as being 0.05% to 0.2% of unselected renal neoplasms. In the local case series from Australia (Sydney), we found no morphologically or immunohistochemically compatible cases in 420 consecutive unselected renal tumors screened both by morphology and IHC. Similarly, only 1 and 2 cases were identified in large population-based cohorts of 2004 and 1750 consecutive renal carcinomas, respectively, in institutions from Europe (Pilsen) and North America (Calgary), which were screened by morphology. A limiting factor in the 2 latter series was the lack of systematic IHC for SDHB and SDHA, which could have potentially detected additional cases, particularly any with variant morphology. However, identification of the cases in these cohorts

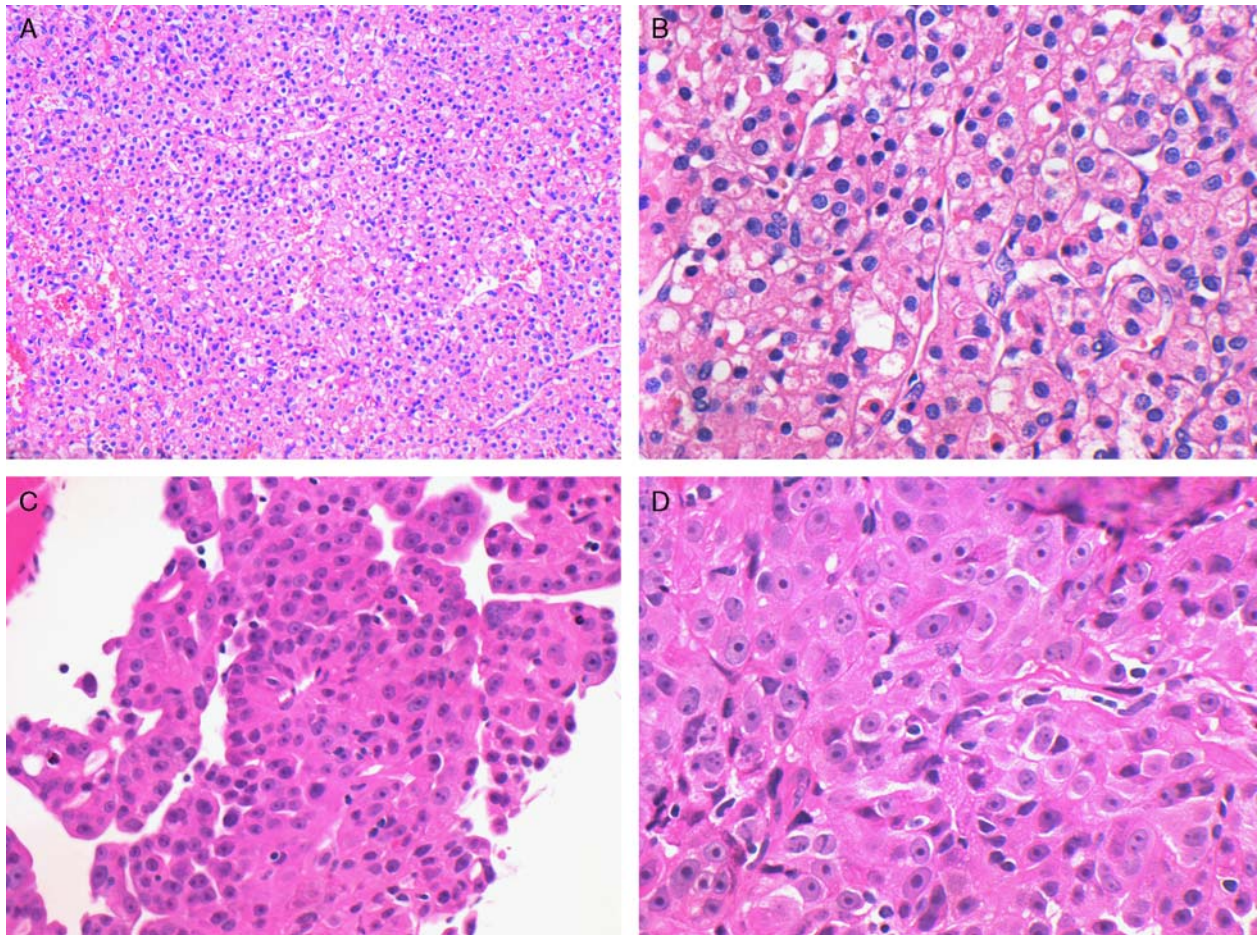


FIGURE 7. Representative photomicrographs from the primary tumor (A and B) and the vertebral metastasis (C and D) of case 10. The primary tumor demonstrated stereotypical low-grade features with ISUP nucleolar (nuclear) grade 2. In the metastasis documented 30 years later, the tumor demonstrated high-grade nuclear features but still showed negative staining for SDHB. As the patient had a contralateral renal tumor, which was unbiopsied at the time of metastatic disease, this may represent spread from a second primary tumor (hematoxylin and eosin).

was based on the recognition of an unusual morphology and routine IHC, in the setting of large centralized uropathology practices with experienced genitourinary pathologists. Thus the estimated incidence derived from 3 institutions from different continents was similar and ranged from 0.05% to 0.2%. These results are also in keeping with the recently reported data by Miettinen et al,⁴² who performed IHC on 711 renal carcinomas and 64 oncocytomas and found that only 4 cases (0.5%) demonstrated loss of staining for SDHB.

The low incidence of SDH deficiency in renal carcinomas is similar to the low incidence reported in pituitary adenomas (0.3%),¹⁵ and contrasts to the high incidence found in pheochromocytoma/paranglioma (3% in adrenal pheochromocytomas and up to 40% in extra-adrenal parangliomas)⁶ and significant incidence in gastric GIST (5% to 7.5%).^{3,10} Therefore, although it has been recommended that all pheochromocytomas and all parangliomas as well as gastric

GISTs with compatible morphology for SDH-deficient GIST undergo screening IHC for SDHB,^{1,6,18} it is unlikely to be cost-effective or practical to screen all renal carcinomas with SDHB IHC.

This study confirmed that classical low-grade tumors showing typical histologic features and an ISUP nucleolar (nuclear) grade 2 are usually cured by excision alone. Of the 9 patients who developed metastatic disease, in only 2 did the primary tumor demonstrate exclusively low-grade features with an ISUP nucleolar (nuclear) grade 2. Importantly, by the time of metastasis, both of these patients had developed a contralateral renal neoplasm, which had not been resected or biopsied. Therefore the metastasis may have arisen from the metachronous tumors, which may have been of higher grade and not from the primary low-grade SDH-deficient renal tumor.

We note that SDH-deficient renal carcinoma may undergo dedifferentiation including sarcomatoid transformation, and cases with high-grade nuclei commonly

metastasize. In fact, metastatic disease developed in 7 of 10 patients with ISUP nucleolar (nuclear) grade 3 or 4 nuclei or variant morphology. Although hemorrhage, fibrosis, and hyalinization were relatively common, only 4 tumors demonstrated true coagulative necrosis. Given that all 4 of these metastasized (and 3 were confirmed dead of disease), it is likely that coagulative necrosis is an adverse prognostic indicator.

Given the low risk for metastatic disease and the high incidence of bilateral tumors in 7 of 27 (26%) patients, our findings support nephron-sparing surgery for patients with low-grade tumors. Although there is insufficient evidence to recommend adjuvant treatment, patients with high-grade neoplasms (variant morphology, sarcomatoid change, coagulative necrosis, or high ISUP nucleolar [nuclear] grade) should be considered at high risk for metastasis, and consideration should be given to more radical treatments in these patients. We note that in 2 patients metastasis occurred >5 years after the initial presentation, and therefore extended (if not lifelong) follow-up is required for late recurrences, as well as metachronous disease and other syndromic manifestations of germline *SDH* mutation (GIST, paraganglioma, pituitary adenoma).¹

The differential diagnosis of SDH-deficient renal carcinoma, which includes oncocytoma and chromophobe renal carcinoma is limited, and we consider loss of staining for SDHB as definitive confirmation of the diagnosis. Although SDHB IHC is not widely available, the morphologic features of typical SDH-deficient renal carcinoma, such as uniform low-grade morphology in the great majority of cases, flocculent (rather than truly oncocytic) cytoplasm, cytoplasmic vacuoles, lack of distinct cell borders, negative staining for c-KIT, and commonly negative or focal cytokeratin reactivity, are important clues to the diagnosis.

In our series of SDH-deficient renal carcinomas, germline mutations were identified in all 17 patients who underwent genetic testing. This is similar to the findings in SDH-deficient paragangliomas and pituitary adenomas, where the presence of negative staining for SDHB almost always signifies germline mutation of one of the components of the mitochondrial complex 2 (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*), rather than being due solely to somatic inactivation.¹ In fact, we are aware of only 2 cases of SDH-deficient paraganglioma and 1 case of SDH-deficient pituitary adenoma in which double-hit SDH inactivation has occurred in the absence of germline mutation.^{15,44,45} It is possible that our series is subject to a referral bias because patients with known mutation or personal or family histories of syndrome-related tumors were more likely to be recognized and included in this study. However, our findings suggest that, similar to paraganglioma and pituitary adenoma, it is likely that most, perhaps almost all, SDH-deficient renal carcinomas will be associated with germline mutation of one of the SDH genes. Therefore, the diagnosis of SDH-deficient renal carcinoma can be considered an absolute indication for germline *SDH* mutation testing. No clear-cut genotype-phenotype correlations have emerged,

although it is interesting to note in this series that 4 unrelated patients who developed renal carcinoma all harbored the same SDHB [c.423 + 1G > A] splice site mutation and that 2 of the patients with this mutation developed multifocal disease.

Although SDH-deficient renal carcinoma shows an extremely strong correlation with germline SDH mutation, we believe that IHC remains a phenotype test rather than a genotype test, and it is likely that not all SDHB IHC-negative tumors will be shown to have SDH mutations using current technology. Therefore, as we have previously stated in the setting of paraganglioma,⁶ we do not believe that specialized consent or formal genetic counseling would ordinarily be required before IHC is performed. This is analogous to IHC for DNA mismatch-repair proteins being used to triage patients with colorectal cancer for genetic testing for Lynch syndrome wherein there is now a trend toward universal screening, and most jurisdictions do not require genetic counseling before screening IHC is performed.

To date, no mutations in *SDHA* have been reported in association with renal carcinoma, but given that loss of staining for SDHA identifies both paragangliomas and GISTs associated with germline *SDHA* mutation,^{1,18–20,46–48} we would recommend that IHC for SDHA also be performed in SDH-deficient renal carcinoma to assist in triaging genetic testing for *SDHA* mutation.

The extremely high rate of germline mutation in the SDH subunits in SDH renal carcinoma is different to that found in SDH-deficient GIST, in which approximately 30% of cases are associated with *SDHA* mutation, and 10% to 20% of cases are associated with mutations in the other SDH subunits (*SDHB*, *SDHC*, or *SDHD*), leaving the mechanism of SDH deficiency uncertain in up to half of cases.^{18,19,46–48} It is noteworthy that some patients with SDH-deficient GIST but without germline mutation were found to have the Carney Triad (the nonhereditary but syndromic association of SDH-deficient GIST, paraganglioma, and pulmonary chondroma).³ It is therefore possible that some patients with SDH-deficient renal carcinoma may be syndromic, even if no germline mutations are identified. From a practical point, because long-term follow-up is required due to the possibility of late metastasis, we would also recommend long-term follow-up for other syndromic manifestations (pheochromocytoma/paraganglioma, GIST, pulmonary chondroma, or pituitary adenoma), regardless of whether or not a germline mutation is identified. In fact, although there may have been a selection bias toward recognizing patients with syndromic disease, we note that in our series 30% of patients also developed either paraganglioma or SDH-deficient GISTs—a particularly striking association given the relative rarity of these tumors.

In conclusion, SDH-deficient renal carcinoma represents a distinct and rare renal neoplasm, which is defined by loss of IHC staining for SDHB. Because of its rarity, it is impractical to perform reflex screening IHC on all renal cancers. However, the great majority of SDH-deficient

renal tumors (94% in this series) demonstrated typical appearances at least focally and were recognized by their uniform low-grade cytology, cytoplasmic vacuoles, eosinophilic or flocculent (rather than truly oncocytic) cytoplasm, focal cystic change, and solid to lobulated growth with peripherally entrapped renal tubules. In tumors exhibiting low-grade nuclear features with ISUP nucleolar (nuclear) grade 2, metastasis is unusual but can occur even after a prolonged period. SDH-deficient renal carcinoma may be associated with high ISUP nucleolar (nuclear) grade, coagulative necrosis or sarcomatoid transformation, in which case the development of metastatic disease is much more likely. SDH-deficient renal carcinomas are commonly multifocal and with prolonged follow-up, bilateral tumors can be identified in up to 26% of patients. To date, all reported cases have been associated with germline mutations of the *SDH* genes.

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